

hydrogenolysis. The type of bonding and linkage of pollutants/metabolites can be better elucidated using isotope-labelled Na^{18}OH , because only the carboxylic entity of the ester carries the heavier oxygen isotope, thus referring to products attached to the HOM matrix by ester bonds.

Although wet chemical degradation techniques as well as pyrolysis techniques involve some pitfalls and limitations, they can contribute to elucidation of the fundamentals of the diagenesis of organic compounds in soil and water. ^{13}C NMR results yield strong evidence that a large percentage (in general, about 60% in case of the HOM under study) of methyl groups is attached to hydrogen-free paraffinic carbon. These findings cannot be explained by building blocks consisting of alkanes or alkylbenzenes. Steranes and hopanes, considered to be the end products of a complex web of diagenetic reactions starting from functionalized bacteriohopanepolyols and serving as biomarkers in ancient sediments and petroleum, are assumed to account for that. Indeed, pentacyclic terpanes with 32–35 carbon atoms, confirmed by tracing $m/z = 191$ in the GC-MS mode, can be detected using both chemical and thermal degradation.

Future Developments

Conventional and TMAH pyrolysis, as well as controlled chemical degradation methods, applied together in combination with highly efficient GC-MS can give insight to the building blocks and the linkages between them in the polymeric HOM network. However, analytical pyrolysis has a significantly higher potential in revealing the chemical nature of simpler polymers, e.g. polyethylene and polystyrene, which give no or minor solid residue. Traditionally performed pyrolysis work with HOM conceals much significant information on the chemical nature of HOM by thermal degradation of functional groups and thermal rearrangements. Thermochemolysis with TMAH gives rise to complementary and more independent reactions in comparison

with conventional pyrolysis. Not much attention has been paid to improved specificity of bond cleavage in the structures. It will be important to understand the mechanisms of the cleavages, and to be able to relate the products identified to possible structures in the parent macromolecule.

See also: II/Chromatography: Gas: Derivatization; Detectors: Mass Spectrometry; Pyrolysis Gas Chromatography.

Further Reading

- Abbt-Braun G, Frimmel FH and Schulten HR (1989) Structural investigations of aquatic humic substances by pyrolysis-field ionisation mass spectrometry and pyrolysis-gas chromatography/mass spectrometry. *Water Research* 23: 1579.
- Hautala J, Peuravuori J and Pihlaja K (1998) Organic compounds formed by chemical degradation of lake aquatic humic matter. *Environment International* 24: 527.
- Hayes MHB, MacCarthy P, Malcolm RL and Swift RS (1989) The search of structure: setting the scene. In: Hayes MB *et al.* (eds) *Humic Substances II*, chap. 1, pp. 1–31. John Wiley.
- Kopinke FD and Remmler M (1995) Reactions of hydrocarbons during thermodesorption from sediments. *Thermochimica Acta* 263: 123.
- Lighthouse E (1998) Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soil. *Naturwissenschaften* 85: 76.
- Poerschmann J, Kopinke FD, Balcke G and Mothes S (1998) Pyrolysis pattern of anthropogenic and natural humic organic matter. *Journal of Microcolumn Separations* 10: 401.
- Richnow HH, Seifert R, Hefter J *et al.* (1997) Organic pollutants associated with macromolecular soil organic matter: mode of binding. *Organic Geochemistry* 26: 745.
- Saiz-Jimenez (1996) The chemical structure of humic substances: recent advances. In: Picollo A (ed.) *Humic Substances in Terrestrial Ecosystems*, ch. 1, pp. 1–44. Amsterdam: Elsevier.
- Schulten HR and Leinweber P (1996) Characterisation of humic and soil particles by analytical pyrolysis and computer modelling. *Journal of Analytical and Applied Pyrolysis* 38: 1.

Liquid Chromatography

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Humic substances are complex mixtures of compounds and in order adequately to elucidate and

characterize the properties and reactions of humic substances, separation techniques are an absolute requirement. Initial work in this area employed low pressure liquid chromatography (LC) with extensive use of size exclusion chromatography (SEC). The advent and acceptance of high performance liquid

chromatography (HPLC) as well as its miniaturization and the development of column technologies, throughout the 1970s, has resulted in HPLC becoming the most widely used chromatographic technique for the separation of humic substances. The focus here will be on the HPLC of humic materials with only a passing reference to low pressure chromatographic methods.

The physical and chemical properties of humic substances are of obvious importance in their interaction with column materials and subsequent separation in chromatography. A brief description of germane humic characteristics will be presented first, followed by a discussion of the various chromatographic modes of separation, the important detectors employed and new horizons in the LC of humic substances.

Humic Properties

Humic substances are known by many names and can be found in measurable concentrations in almost every soil, water or sediment system on earth. Carbon-containing compounds are the common denominator from both plant and animal sources which break down under the normal sequence of death and microbial decomposition in the environment. All forms of organic carbon (OC) or organic matter (OM) that are dissolved and of natural origin are of interest here and form the broadest category, of which humic substances (HS) or the synonymous humic materials (HM) are the largest part. Nonhumic compounds under the OC classification include identifiable species such as amino acids, sugars, fatty acids and the like. Humic acids (HA) and fulvic acids (FA) are the two subunits of humic substances making up all water-soluble material in this class. The relationship between all of these categories is depicted in Figure 1.

Humic substances are macromolecular species ranging in molecular weight from about 1 to 100 kDa. They have historically not been considered polymeric in nature, lacking any confirmed monomeric unit, although this view has been challenged in recent years with the proposal of an approximately 700 Da monomer. Figure 2 shows a molecular model of the lowest energy building block conformation computed for a humic acid hexamer. Humic substances exhibit significant polydispersity and are polyelectrolytic, containing numerous carboxylic acid and ionizable phenolic groups. Very small amounts of amine functionality are sometimes present; however, essentially no sulfur groups are found associated with humic samples. Properties of humic substances that are relevant to chromatographic sep-

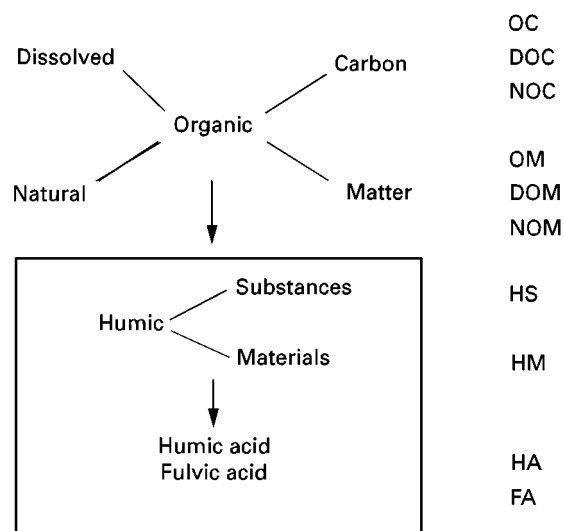


Figure 1 Terminology, acronyms and relationship for soluble organic carbon (OC) of natural origin. Humic and fulvic acid are the two categories of soluble humic substances or humic materials which are a subset of all organic materials in the environment.

arations are summarized in Table 1. Significant among these are the hydrophobicity or surface activity of humic substances causing them to adsorb and partition with appropriate materials, their acidity and polyelectrolytic character imparting water solubility and charge to the molecules allowing for ion exchange, and their range of molecular sizes and possibly shapes that makes feasible separations based on size.

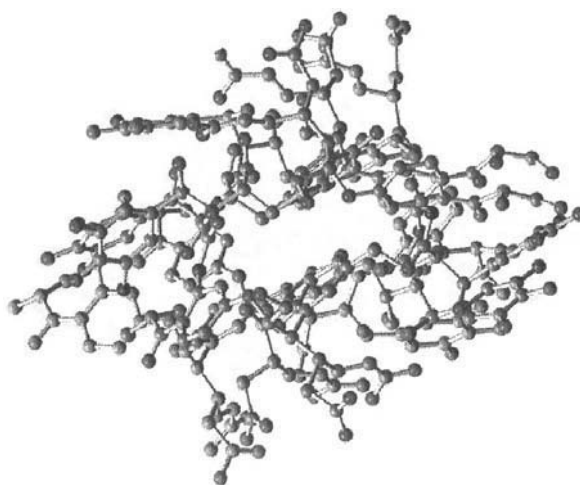


Figure 2 (See Colour Plate 87). Molecular model of the lowest energy conformations of humic acid building blocks linked to form a hexamer. Carbon atoms are green, oxygen atoms are red, nitrogen atoms are blue and hydrogen atoms are not shown. Reproduced with permission from Davies and Ghabbour (1999).

Table 1 Summary of characteristics for typical humic substances

Elemental composition	Approximately 50% C, 5% H, 45% O; very little N, no S
Ash content	Typically less than 0.5%
Molecular weight range	1–100 kDa
Purity	Complex mixture
Structure	Difficult to specify exactly
Functional groups	Aromatic and aliphatic carboxylic acids Phenolic OH and aliphatic OH Carbonyl groups Numerous aromatic rings Aliphatic chains Quinone/hydroquinone present Traces of bound metals, particularly Fe and Al
Acidity	Approximately 6 mmol g ⁻¹ from carboxyl groups
Solubility	Very soluble in water; generally, solubility increases with pH Poor solubility in most organic solvents
Other characteristics	Polydisperse Polyelectrolytic Surface active: hydrophobic portion with hydrophilic groups Metal complexation: binding of numerous metal ions Binding of organic molecules

Chromatographic Modes

Size Exclusion Chromatography

Some of the earliest LC separations of humic substances were based on the size exclusion mode of separation using distilled water, salt solutions or aqueous buffers as mobile phases. SEC studies showed that size separations were possible but, unfortunately, most attempts at SEC of humic substances gave poor resolution and marginal results. Chromatograms typically exhibited only one or two broad peaks with an occasional shoulder. Experiments resulting in several peaks by SEC have often been found by subsequent analysis to be the result of a mixed-mode separation. This comes about in low ionic strength mobile phases because of the repulsive forces between negatively charged humic substance molecules and negative charges on the surface of the column-packing materials. The actual separation is based partially on size and partially on charge, resulting in poor reproducibility and dramatic changes in the chromatography with slight changes in ionic strength, pH or solution composition. This complication completely eliminates the possibility of obtaining any molecular weight information from size exclusion – something that is normally a strong point of the method.

Once the problems with SEC of humic substances were better understood, improved packing materials such as the TSK gels (Toyo Soda, Tokyo, Japan) provided somewhat better resolution and reduced surface charge. However, the SEC of humic substances did not improve dramatically with these materials, primarily because of the nature of the humic substances themselves. Although humic substances have a seemingly broad size range, most samples have been preselected for size because of environmental conditions or by a particular isolation procedure, i.e. the method by which the sample was extracted from soil, sediment or water. In addition, each humic sample may contain a fairly uniform distribution of molecules over that range. These factors result in simple one- or two-peak chromatograms consisting of broad or poorly resolved peaks, such as those shown in **Figure 3**. The recent literature on humic substances has revealed little application of the SEC technique.

Ion Exchange

Ion exchange chromatography has found fairly limited application in the separation of humic

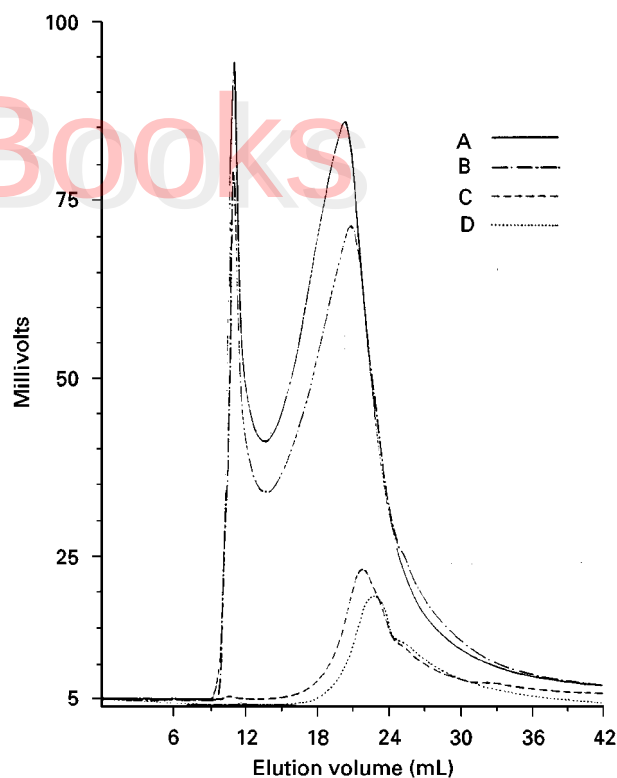


Figure 3 Size exclusion chromatograms of a humic acid sample on a TSK G3000SW (600 × 7.5 mm i.d.) with UV detection at 280 nm. Mobile-phase conditions are as follows: A, 0.05 mol L⁻¹ NaNO₃, pH 7; B, same as A with 4.6 × 10⁻⁷ mol L⁻¹, pH 6.97; C, same as A with pH adjusted to 5.54 with HCl; D, same as A with 4.6 × 10⁻⁷ mol L⁻¹ acetic acid, pH 5.69. Reproduced with permission from Conte and Piccolo (1999).

substances, possibly because of the very strong retention of humic molecules for most ion exchange packings. Early work with diethylaminoethyl (DEAE) functionalized supports demonstrated that ion exchange of humic substances is feasible, but rather harsh conditions must be used to approach quantitative elution of the retained material from the column. Some researchers advocated the use of 0.5 mol L^{-1} NaOH to obtain the best recoveries of humic substances from DEAE columns. Concern about the possible alteration of the humic substance molecules (hydrolysis, etc.) under these conditions has restricted its use. Ion exchange separations have been used extensively in the past for isolation and purification of humic substances from the environment. However, strongly basic conditions are also required for elution in this application and there has been a concerted effort in the area of humic substance isolation and purification to move towards less severe methods.

Reversed-phase HPLC

The general popularity of the reversed-phase mode for HPLC (RP-HPLC) separations in analytical chemistry is paralleled by its prominence as the chromatographic mode most applied to humic separations. One important reason for the development of this situation is the availability of several variables that can be adjusted to influence the separation. The percentage of organic modifier such as methanol, 2-propanol or acetonitrile can be regulated and held constant in isocratic separations or varied (either stepwise or continuously) in gradient elution. Ionic strength and buffer pH are commonly adjusted to improve resolution and even the length (C_1 to C_{18}) or type of stationary phase can be changed from alkyl to phenyl, diol or other types of functionality.

With all the flexibility available in RP-HPLC separations, it is often unclear upon initial examination why essentially all of the published chromatograms of humic substances exhibit broad, poorly resolved peaks that by some chromatographic standards would be unacceptable. The reason for this dilemma once again is linked to the nature of the mixture of molecules in humic substances. One view suggests that, although varied in properties, the molecules form a near continuum of species with characteristics so similar to one another that they are difficult to separate. The appearance of isolated peaks in the chromatogram is a function of greater numbers of molecules of certain types (in the continuum) over other molecules which elute in the valleys between peaks.

Since RP-HPLC separates on the basis of polarity, most chromatograms show two or three distinct re-

gions of peaks. Early in the chromatogram the most polar compounds elute, often as a jumble of sharp, but unresolved bands. Late in the chromatogram, the nonpolar species appear usually as very broad peaks with unresolved shoulders or side bands. Sometimes, a band of intermediate polarity is present midway through the chromatogram.

An important consideration in selecting a column for separating humic substances by RP-HPLC is the pore diameter of the packing material. Reversed-phase supports are available with a variety of pore sizes; however, larger pore diameters are more suitable for larger molecules such as humic substances. A common choice is a 30 nm pore diameter sold as a reversed-phase column for protein separations.

Ion Pair Reversed-phase Chromatography

Improvements in the RP-HPLC separation of many charged species can be realized by adding an ion-pairing reagent to the mobile phase. The ion-pairing reagent is commonly a tetrabutylammonium salt (in the cationic case) which can form an ion pair with the species of interest. Humic substances contain many ionizable carboxylic acid and phenolic groups, making them very suitable for this mode of chromatographic separation. **Figure 4** shows a representative ion pair reversed-phase (IP-RP-HPLC) chromatogram for the fulvic acid fraction of humic substances derived from soil. Although IP-RP-HPLC represents some improvement over RP-HPLC, the nature of the humic substances still gives rise to broad peaks that are less than completely resolved.

With regard to the exact mechanism of the separation in IP-RP-HPLC, at least two models exist. One perspective is that the ion pairing alters the polarity of the humic substance molecules, dramatically changing their retention characteristics on the reversed-phase column. A second theory supposes that the ion-pairing reagent partitions into the column leaving charged sites that allow for an ion exchange process with the humic substances as a means of separation. Reality is most probably somewhere between these two viewpoints. It should be noted that, once a RP-HPLC column has been subjected to an ion-pairing reagent, it is always an ion-pairing column.

Table 2 gives an overview of the HPLC modes discussed above. Representative publications are included for further reading in this area.

Detectors

Humic substances absorb UV and visible radiation at essentially all wavelengths and can therefore be readily monitored by absorbance detectors in HPLC. The

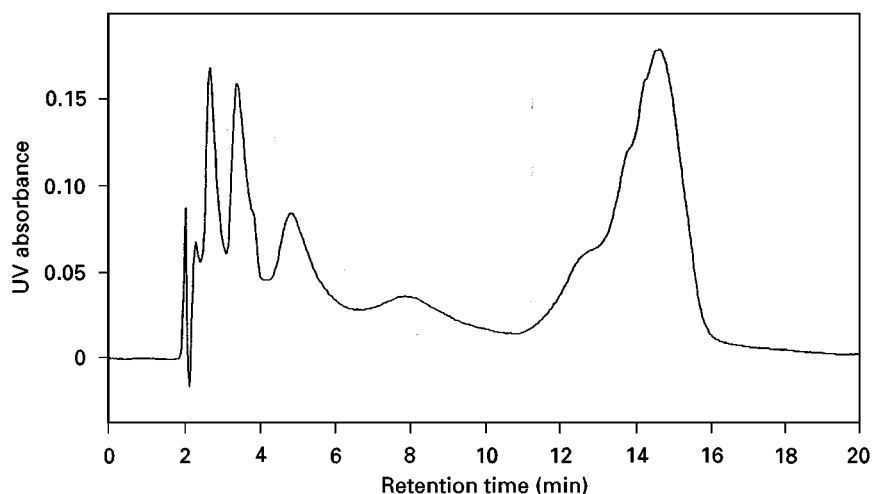


Figure 4 Ion pair reversed-phase chromatogram of Swanee Stream reference fulvic acid (SSRFA) separated on an SEG C₁₈ column using gradient elution beginning with 37% CH₃CN for 8 min followed by a linear gradient to 68.5% CH₃CN over 8 min at a flow rate of 1 mL min⁻¹. Tetrabutylammonium perchlorate was used as the ion-pairing reagent at a concentration of 50 mmol L⁻¹. UV absorbance at 254 nm was monitored for an 8 μ L injection of 0.048% (w/v) SSRFA.

absorbance spectrum of a typical humic substance sample is essentially featureless, looking somewhat like an exponential curve. Absorbance is high in the short wavelength region of the UV spectrum and drops quickly with increasing wavelength, tailing off into the red region of the visible spectrum. Therefore, almost any wavelength can be used to detect humic substances, but shorter wavelengths are more sensitive. Fixed wavelength measurements at 254 nm are the most common; however, variable wavelength detectors have been used at a variety of wavelengths with good success.

Photodiode array (PDA) monitoring of the complete UV-visible spectrum of the column effluent adds an additional dimension to HPLC, providing several advantages over conventional single wavelength detectors. PDA systems repeatedly collect spectra at a specified time interval (often once every second) throughout the life of the chromatogram. This spectral information is invaluable in method development, particularly when many unknown

compounds are present. Once the data are collected, several chromatograms can be generated, each with a different monitoring wavelength revealing features not visible in a single wavelength chromatogram. For example, a single well resolved peak at one wavelength may show a poorly resolved doublet at another wavelength. Similarly, peak purity can be determined by examining the spectra at several time intervals across a peak.

Finally, PDA detection can aid in the qualitative identification of the separated components by comparison of a peak's spectrum with the spectrum of known compounds. This approach has been used in some applications to elucidate certain structural features in separated fractions of humic substances. The general drawback with this application is that UV-Vis absorbance spectra are not conclusive evidence of a compound's identity and can only provide possible structural clues. When applied to the complex mixture of humic substances, only vague inferences can be made about possible structural components in

Table 2 Modes of liquid chromatography employed for separations of humic substances (HS)

Mode	Mechanism of separation	Reference
Size exclusion	Exclusion of larger HS molecules from certain pores based on size	Chin <i>et al.</i> (1994) Huber and Frimmel (1994)
Reversed-phase	Partitioning of hydrophobic portion of HS molecules into nonpolar stationary phase	Saleh <i>et al.</i> (1989)
Ion exchange	Exchange of anionic HS on cationic stationary phase	Andres <i>et al.</i> (1987)
Ion pair reversed-phase	Retention of ion-paired HS on nonpolar phase or ion exchange of HS on ion-pairing reagent-loaded phase	Butler and Ryan (1996)

the macromolecules that are contained within a peak. This type of data gives further evidence that moieties such as vanillic acid, catechol or other substituted aromatics are part of the structure of humic substances, but cannot provide unequivocal results.

The second and only other major detection method used for the HPLC of humic substances is fluorescence. Humic substances emit a broad fluorescence peak centred around 450 nm when excited by radiation in the 330–350 nm range. This fluorescence is not considered to be a very strong signal (high quantum yield) in comparison to other fluorescent species; however, sufficient sensitivity can be obtained with standard commercial fluorescence detectors to measure easily humic substances down to the low part-per-million level sufficient for most environmental measurements. Fluorescence is a selective means of detection because not all molecules fluoresce – a situation that is clearly true of the mixture of molecules in humic substances. HPLC experiments with both absorbance and fluorescence detection have shown that some components of humic substances absorb but do not fluoresce. This lack of fluorescence may result from one of at least two possibilities. The absence of the appropriate structural features (usually extended π electron system) in certain fractions of humic substances will render them non-fluorescent or the presence of fluorescence-quenching agents in a normally fluorescent fraction will likewise remove fluorescence. The most common fluorescence quenchers for humic substances are the paramagnetic metal ions, such as Cu^{2+} , Fe^{3+} , Ni^{2+} , Co^{2+} , Mn^{2+} , which form complexes at appropriate binding sites on the molecule. Recent studies have also shown that certain organic molecules can bind to humic substances either electrostatically or through hydrophobic interactions and also quench fluorescence.

Other Considerations

Humic substances are a fairly difficult class of compounds to work with because they are poorly characterized with respect to their chemical structure and many of their properties are not yet fully understood. Intra- and intermolecular hydrogen bonding, hydrophobic interactions and electrostatic effects surely play a role in what can be called the tertiary structure or shape of humic substance molecules. The variables of pH and ionic strength clearly play a role in determining molecular shape, which is very important with respect to HPLC separations; however, an understanding of the factors influencing shape is only now yielding to analysis through the use of molecular modelling.

Irreversible adsorption, concentration effects and solubility problems also plague the analysis of humic substances by HPLC. Although the fulvic acid fraction of humic substances is soluble at any pH, humic acid is not soluble at low pH and may have varying solubility depending on the exact solution conditions. Samples of isolated humic substances must be carefully dissolved and filtered before HPLC analysis and even then may not remain soluble throughout the separation because of changing gradient conditions.

Concentration effects are a related problem since high concentrations tend to favour aggregation and coagulation of humic substances, resulting in precipitation of the most nonpolar components in the sample. An important consideration when examining the published results in chromatographic studies of humic substances is the concentration of the solution injected into the chromatographic system. Many studies are conducted using as much as 500–1000 mg L⁻¹ of humic substances – a concentration level that is orders of magnitude higher than typical environmental levels in most cases. The results obtained in these instances may be influenced by concentration artifacts and are not likely to be representative of environmental conditions.

Irreversible adsorption is another difficulty that frequently occurs at the top of guard or analytical columns because of a high affinity of the column packing material for the humic substances. This mechanism may result in significant losses of sample material to the column and may skew results by selectively removing some compounds while not affecting others. The presence of bare silica for silica-based packings or other active sites can influence adsorption. In addition, the presence of sample components or impurities such as metal ions can facilitate interactions between humic substances and active sites on a column.

New Horizons

Although HPLC may be considered a mature technique, many advances have been made in recent years, primarily in the area of detectors, that have an impact on the chromatography of humic substances. One example is the use of carbon analysers as detectors for HPLC. Instruments that measure organic carbon in solution have long been used for the measurement of humic substances and other natural organics in nonchromatographic applications. It is only in the last decade that these instruments have been modified for use in HPLC. The advantages of dissolved organic carbon (DOC) detectors include their universal detection of all organic compounds

and their specificity for only carbon-containing species.

Two new approaches in the area of fluorescence detection of humic substances are the coupling of a fluorescence lifetime instrument to HPLC and post-column fluorescence quenching titrations as a means of measuring metal complex equilibria. Fluorescence lifetime measurements add yet another means of increasing the specificity of analysis, allowing improved qualitative determinations and generally increasing the amount of information obtained from fluorescence analysis of a sample. The coupling of phase modulation spectrofluorometry to HPLC results in a powerful method for measuring and resolving overlapping peaks as well as adding a dimension for identifying unknowns in a chromatogram. Although this type of detector is inherently expensive and will not see routine use, its application to humic substances, which have been shown to have three distinct fluorescence lifetimes, seems logical.

Post-column fluorescence quenching titration experiments have recently been employed in metal complexation studies to elucidate binding of metals to fractions of humic substances separated by HPLC. Quenching of the natural fluorescence by certain metal ions has become an accepted method for measuring equilibrium constants and other binding parameters for complexation of the metal by sites on humic substance molecules. Studying these equilibrium processes by HPLC has heretofore been very difficult because the chromatographic separation of the humic-metal complexes is adversely influenced by the presence of the metal ion. By separating the humic substances first without metal present, then adding metal ion via a post-column inline mixing tee, the complexes are formed just prior to their measurement by a conventional fluorescence detector. This approach alleviates any chromatographic problems associated with the presence of metal ions and allows valuable binding studies of metal ions with separated fractions of humic substances in a time-saving online mode.

Two of the most powerful instruments for measuring chemical structures of organic molecules are Fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS). A relatively new form of MS that has a major advantage in its application to macromolecules such as proteins is matrix-assisted laser desorption and ionization (MALDI) MS. The immense benefit of coupling a powerful separation technique such as HPLC with the unparalleled structural capabilities of FTIR and MS is obvious, particularly when applied to complex mixtures. However, there are significant difficulties encountered in the interfacing of these techniques which have hindered

advances in this area. A novel approach has been developed to meet this challenge which centres on the inherent ability of FTIR and MS to analyse solid samples. Column effluent which exits from the chromatograph is sprayed on to a slowly moving solid substrate under controlled conditions. Solvent is evaporated and the sample dried as the separated components are deposited sequentially. Once the chromatographic run is complete, the solid substrate with the deposited sample components is placed in a modified FTIR or MALDI MS unit for analysis. Sequential analysis along the track of deposited sample components yields a series of spectra similar in concept to PDA detection in HPLC, but this approach is far more useful in elucidating structural features and providing absolute qualitative confirmation of component's identity. The application of this technology to the analysis of humic substances with RP-HPLC has been pioneered.

Conclusion

The use of HPLC for separation of humic substances has been, and will continue to be, a challenging area in separation science. Additional information about the nature of humic substances will aid in the fine-tuning of this application of HPLC, as will improvements in column technology. Probably the most significant advances in the HPLC of humic substances will come with the utilization and development of more powerful detectors, particularly spectroscopic detectors. No doubt the complex, heterogeneous nature of humic substance samples will require a host of separation and detection strategies to be applied in the years to come before their analysis is considered in any way routine.

See Colour Plate 87.

See also: II/Chromatography: Liquid: Detectors: Fluorescence Detection; Detectors: Ultraviolet and Visible Detection; Ion Pair Liquid Chromatography; Mechanisms: Reversed Phases; Mechanisms; Size Exclusion Chromatography.

Further Reading

- Andres JM, Romero C and Gavilan JM (1987) Ion exchange chromatography of fulvic acids from lignite. *Fuel* 66: 827.
- Butler GC and Ryan DK (1996) Investigation of fulvic acid-Cu²⁺ complexation by ion-pair reversed-phase high-performance liquid chromatography with post-column fluorescence quenching titration. In: Gaffney JS, Marley NA and Clark SB (eds) *Humic and Fulvic Acids: Isolation, Structure and Environmental Role*, p. 140. Washington, DC: ACS Symposium Series 651.

- Chin YP, Aiken G and O'Loughlin E (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science and Technology* 28: 1853.
- Conte P and Piccolo A (1999) Conformational arrangement of dissolved humic substances. Influence of solution composition on association of humic molecules. *Environmental Science and Technology* 33: 1682.
- Davies G and Ghabbour EA (1999) Understanding life after death. *Chemistry and Industry* 7 June: 426.
- Huber SA and Frimmel FH (1994) Direct gel chromatographic characterization and quantification of marine dissolved organic carbon using high-sensitivity DOC detection. *Environmental Science and Technology* 28: 1194.
- Liu X and Ryan DK (1997) Analysis of fulvic acids using HPLC/UV coupled to FTIR spectroscopy. *Environmental Technology* 18: 417.
- Ryan DK, Thompson CP and Weber JH (1983) Comparison of Mn^{2+} , Co^{2+} , and Cu^{2+} binding to fulvic acid as measured by fluorescence quenching. *Canadian Journal of Chemistry* 61: 1505.
- Saleh FY, Ong WA and Chang DY (1989) Structural features of aquatic fulvic acids. Analytical and preparative reversed-phase high-performance liquid chromatography separation with photodiode array detection. *Analytical Chemistry* 61: 2792.
- Sein LT, Varnum JM and Jansen SA (1999) Conformational modeling of a new building block of humic acid: approaches to the lowest energy conformer. *Environmental Science and Technology* 33: 546.
- Smalley MB, Shaver JM and McGown LB (1993) On-the-fly fluorescence lifetime detection in HPLC using a multiharmonic Fourier transform phase-modulation spectrofluorometer. *Analytical Chemistry* 65: 3466.

HYDRODYNAMIC CHROMATOGRAPHY: PRACTICAL APPLICATIONS



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Introduction

Either in research, the control or the production of chemicals, or during general observations in biochemistry, characterization is a primary necessity. Depending on the product, the nature, structure, size, shape, and molecular weight are some of the important parameters to be measured. For instance, the molecular weight distribution of polymers and particle size of latex/colloids (MWD and PSD, respectively) have to be known so that they can be correlated with properties. The data may be obtained using many techniques. These techniques are governed according to the various properties of a material and depend on the size range of the investigated material. Hydrodynamic chromatography (HDC) is one of these techniques and has found applications for sizing soluble or dispersed solid components. This article will discuss the size distribution of organic latex particles from the theoretical and practical points of view. Progress in packing columns with fine materials or in the use of fine capillary tubes has allowed rapid separation of species with high resolution. A second field of interest is polymers in solution. The combined effects of hydrodynamic and exclusion chromatography have extended possibilities for the separation of high-molecular-weight materials.

Hydrodynamic chromatography is used for diameter determination in the micron range (some nm to some μm). It will work for both solid and soluble samples, which are eluted according to their decreasing size. This leads to a visual picture of the size distribution. The main interest of HDC lies in the rapid separation (fractionation, which is an alternative name for this method: HDF) of the liquid or solid components present in the sample. Often low peak capacity and poor resolution are its limitations and involve the necessity for peak dispersion correction. Moreover, a quantitative study requires a double calibration. The first relates to elution volume and diameter of the analysed particles; the second gives a correspondence between the signal intensity and size of particles. This intensity depends on the nature of the detector and the operating conditions, for instance the choice of wavelength in UV. As an example, **Figure 1** shows the different absorbance curves of polystyrene (PS) latexes of different sizes. **Figure 2** shows the change in absorbance and scattering with wavelength and illustrates different peak contributions of PS particles to the chromatogram.

HDC operates similarly to size exclusion chromatography (SEC) and field flow fractionation (FFF), but needs one (inert) mobile phase and one (hydrodynamic) field only. The interesting principle of particles separation is the difference in their transport rates in a capillary, related to their location in the eluent. Large particles are preferentially in the centre of the capillary, where the flow rate is max-